The University of Chicago

REACTION OF LYMPHATIC TISSUE IN EARLY STAGES OF BACTERIUM MONOCYTOGENES INFECTION

A PART OF A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

1936

By

ELEANOR A. CONWAY

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REACTION OF LYMPHATIC TISSUE IN EARLY STAGES OF BACTERIUM MONOCYTOGENES INFECTION

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The isolation of Bacterium monocytogenes by Murray, Webb and Swann [1] promised to be of great aid in clearing up much of the controversy which has dominated the question of the origin of the monocytes of the blood, for when appropriate doses of a culture of this bacterium are injected into rabbits, monocytosis ensues in the blood stream with great regularity. In view of this it seems that a careful histologic analysis of the organs and viscera of these rabbits should disclose the site of origin and the cells from which the monocytes develop. But this has not been the case, for the several investigators who have used this bacterium to study the precursor of the monocyte offer divergent views on the origin of this cell (Murray, Webb and Swann;[1] Bloom;[2] Lang;[3] Nyfeldt;[4] Rezzesi;[5] Wallbach;[6] Levi and Penati[7]). The details of their reports are considered in this paper in the comment; here it is only necessary to point out that all of these investigators failed to examine the tissues of their infected animals before the number of monocytes in the circulating blood had increased.

In an attempt to determine the source of the monocytosis and to explain some of the conflicting reports on the effects of the injection of this bacterium, I have studied the blood and organs of a series of rabbits which were infected with portions of the same cultures of the

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1. Murray, E. D. G.; Webb, R. A., and Swann, M. B. R.: J. Path. & Bact. **29**:407, 1926.

2. Bloom, W.: Folia haemat. **37**:1, 1928.

3. Lang, F. J.: Folia haemat. **36**:383, 1928.

4. Nyfeldt, A.: Folia haemat. **47**:1, 1932.

5. Rezzesi, F. D.: Haematologica **14**:239 and 287, 1933.

6. Wallbach, G.: Arch. d'anat. micr. **30**:275, 1934.

7. (*a*) Levi, G. M., and Penati, F.: Arch. per le sc. med. **58**:773, 1934; (*b*) Haematologica **16**:317, 1935. (*c*) Penati, F., and Levi, G. M.: ibid. **16**:261 and 409, 1935.

bacterium and which were killed at close intervals in the period from the time of injection until the monocytes were present in the blood in great numbers.

One cannot tell the degree of virulence of this bacterium as it was used by most of the previous investigators. Indeed, there is evidence in their reports that during the course of their experiments the organism showed marked variations in virulence. In order to have the virulence of the bacterium in this and in other studies constant, I decided to use as the criterion of virulence the dosage of bacteria necessary to produce a given degree of monocytosis in the peripheral blood in forty-eight hours. This work was extended to a larger series of guinea-pigs to see if this species also reacts to infection with this bacterium with monocytosis, as Murray, Webb and Swann intimated. Another reason for infecting guinea-pigs was to see the effect of this infection on the Kurloff bodies of the lymphocytes and monocytes.

There is no difficulty in distinguishing the typical monocyte of the blood from cells of all other types with the possible exception of those in Naegeli's *monocytoide Myeloblastenleukämie* and occasional nongranular cells (lymphocytes) of normal blood. This separation can be carried out as easily with unstained cells as in supravital, dry or wet smear preparations or on well stained sections. Unfortunately the precursor of the monocyte lacks morphologic criteria by which it can be separated from the precursors of the granulocytes and from the lymphocytes. The literature contains many claims of the morphologic distinction of "monoblasts" from other stem cells. But the multiplicity of the criteria offered by the different investigators is in itself evidence of the insufficiency of most and, as I believe, of all of them.

In view of the conflicting nomenclatures which have been applied to the constituents of the lymphatic tissue in the lymph nodes and in the spleen it is necessary to define the terms which will be used here. The *lymphatic nodule* is a globular accumulation of lymphocytes within dense or loose, diffuse lymphatic tissue. It may be composed mainly of cells of the same type throughout or it may show variations in structure in its different portions. Sometimes its center may consist mainly of reticular cells or macrophages and few lymphocytes or of fibroblast-like cells; this is a *reaction* center. At other times its central portion may be a focus of rapidly multiplying medium-sized and large lymphocytes; this is a *lymphocytopoietic* center. As will be pointed out later, this focus of proliferating lymphocytes may or may not be surrounded by a limiting zone of smaller, inactive lymphocytes. A more extended discussion of these terms and the necessity for using them will be found elsewhere (Conway [8]).

8. Conway, E. A.: Anat. Rec. **69**:487, 1937.

The conflicting opinions on the meaning of the term "malpighian corpuscle" are due to the fact that this name was given to the white pulp of the spleen as seen macroscopically or with low magnification. Some microscopists now use the term to include the white pulp, i. e., the entire periarterial lymphatic tissue sheath, a view to which I would subscribe but for the fact that most pathologists use the term only for the swellings of the sheath caused by the presence of lymphatic nodules within it. On the other hand there are some who describe and picture the sheath of lymphatic tissue with its enclosed artery in cross-section as the "malpighian corpuscle." It is obvious that in a longitudinal section of the artery and its lymphatic tissue sheath at most two nodules can be seen at any point in the sheath, i. e., one on each side of the artery. In cross-section, however, three or more nodules, if present, can be seen in the sheath. Again, if only one nodule is present in the lymphatic tissue of the sheath at a given point, a cross-section here shows the artery to be eccentric in the sheath in most cases. This is the usual description of the "malpighian corpuscle." In cross-section through a part of the sheath in which no nodules are present the artery is centrally located. Finally, the "central" artery sometimes passes through the center of a nodule.

Accordingly, it seems best to dispense with the term "malpighian corpuscle" until there is a consensus as to its meaning. I shall therefore speak of the white pulp of the spleen as the periarterial sheath of lymphatic tissue and its contained nodules as seen in longitudinal sections and shall specifically designate the description of a region of the sheath which happens to be seen in cross-section.

This report covers only the changes in the peripheral blood, the mesenteric and cervical lymph nodes and the spleens of rabbits and guinea-pigs infected with a single standardized dose of Bact. monocytogenes. The histologic changes in omentum, liver, bone marrow, thymus, thyroid, lung, heart, adrenal and kidney were found to have no direct bearing on the origin of the monocytogenic response and are reported elsewhere. The histologic changes in reinfection of rabbits immunized to Bact. monocytogenes will also be included in another report.

This work was carried out at the suggestion of Dr. W. Bloom.

MATERIAL AND METHODS

The strain of Bact. monocytogenes used in these experiments was strain 58, which was furnished by Dr. E. G. D. Murray. It had not been subjected to animal passage for two years previous to this use and had the morphologic characteristics of the avirulent form; i. e., the culture showed long filamentous rods. This avirulent culture served as the stock strain from which both the strain virulent for rabbits and that virulent for guinea-pigs were obtained.

Three successive passages in young adult rabbits sufficed to raise the virulence to the desired degree; 0.2 cc. of a suspension of the growth of three twenty-four hour dextrose-veal infusion agar slants (p_H 7.2) when injected intravenously into a rabbit produced on the average a monocytosis equivalent to 25 per cent of the total number of leukocytes in the peripheral blood forty-eight hours after the injection. As all of the rabbits showed about the same leukocyte count at similar intervals after the injection of the standard dose, the criterion of the "percentage of monocytes" suffices. The injection of this standardized dose did not kill any of the test rabbits in five days.

The ten rabbits of the experimental series included in this report were all young adult males. They were all subjected to injection on the same day; each received 0.2 cc. of the same bacterial suspension, prepared as described. The injection was made intravenously in the marginal vein of the ear. The rabbits were killed one, three, six, nine, twelve, eighteen, twenty-four, thirty-six, forty-eight and sixty-six hours after the injection. Two uninfected rabbits were killed on the same day as the sixty-six hour infected rabbit.

Another portion of the avirulent stock strain of Bact. monocytogenes was subjected to five successive passages in guinea-pigs weighing about 300 Gm. each. The virulence of Bact. monocytogenes recovered from the spleen and liver of the fifth passage guinea-pig was determined as in the case of the rabbit. In this instance 0.1 cc. of a suspension made from the growth of two twenty-four hour dextrose-veal infusion agar slants when injected intravenously into a guinea-pig also produced on the average a monocytosis equivalent to 25 per cent of the total number of leukocytes in the peripheral blood in forty-eight hours. This standardized dose did not kill any test guinea-pig in ten days. In preliminary experiments it was found that with five times the dose used here the same process occurred as will be described for these guinea-pigs, but the entire response was more intense and more rapid and killed the animals in forty-eight hours.

The sixteen guinea-pigs of the experimental series included in this report were male guinea-pigs weighing approximately 300 Gm. each. As in the case of the rabbits, they were all submitted to injection on the same day, each receiving 0.1 cc. of the same suspension of Bact. monocytogenes, prepared as described. The injection was given in the femoral vein, and the guinea-pigs were killed one, three, six, nine, twelve, eighteen, twenty-four, thirty-six, forty-three and forty-eight hours and three, three and a half, four, five, six and seven days after the injection. One control guinea-pig was killed on the day the injection was made and two on the day the last of the test animals was killed. The series of guinea-pigs used was larger than that of rabbits, because the later stages of the infection in rabbits have been described repeatedly, while the course and histologic picture of experimental infection in guinea-pigs do not seem to have been studied.

White blood counts and wet and dry fixed smears were made from blood taken from the ear vein of each animal just prior to injection and again just before killing. In preliminary experiments which were made to determine the virulence of the bacterium, supravital preparations were made of the peripheral blood and of the hematopoietic organs in addition to the dry and moist fixed smears. But it was soon found that the supravital smears added little to the clearcut information obtained from the moist and dry fixed blood and organ smears and from the sections of the organs. Accordingly, as the latter methods formed a permanent record of the experiment and gave as much information as the supravital films, the supravital method was discarded in this series. In the rabbit series blood was also taken to determine the preinjection titer of agglutinins to Bact. monocytogenes, if any were present. The tissues were removed from all the animals while they

were under ether anesthesia, fixed immediately in Zenker-Helly-Maximow solution, embedded in pyroxylin (nitrocellulose), serially sectioned at 8 microns and stained with hematoxylin-eosin-azure II and by the short Foot method for silver impregnation of reticular fibers. Some of the tissues of each organ removed from the rabbits were also fixed in absolute alcohol for basophil leukocytes. The granules of the basophil leukocytes of the guinea-pig are present in material fixed in Zenker-Helly-Maximow solution and stained with hematoxylin-eosin-azure II.

OBSERVATIONS ON THE PERIPHERAL BLOOD OF INFECTED GUINEA-PIGS

The average white blood cell count before the injection was 6,100 cells per cubic millimeter; the highest count was 7,200 and the lowest 5,400. In the animals killed during the first nine hours following the injection there was marked fluctuation in the white cell count. Then followed progressive leukopenia, reaching 4,100 at thirty-six hours, a fairly normal count, 7,300 at forty-three hours and 6,400 at forty-eight hours, leukopenia, 3,900, during the third day, and an increase to 7,800 on the fourth day. From this time on the white count increased progressively to the seventh day, when it reached 10,650 cells per cubic millimeter.

The monocytes began to increase appreciably at twelve hours, when they were 13 per cent of the total number of leukocytes of the guinea-pig, compared with an average of 3 per cent before the injection. At forty-eight hours they numbered 26 per cent of the total number of leukocytes, while at three and a half days they constituted 40 per cent. Then they decreased progressively to 15 per cent on the seventh day.

The large lymphocytes increased from 3 per cent during the first nine hours to 18 per cent at twenty-four hours, with subsequent oscillations between 12 and 20 per cent during the remainder of the experiment. The number of small lymphocytes rose sharply from a preinjection average of 26 per cent to forty-eight per cent in twenty-four hours, which was followed by a progressive decrease as the monocytes increased; the small lymphocytes decreased to 9 per cent on the third day, during which time the monocytes constituted 40 per cent of the total number of leukocytes.

The average number of heterophil (pseudo-eosinophil) leukocytes before the injection was 68 per cent of the total number of leukocytes. They decreased from 70 per cent at nine hours to 18 per cent at twenty-four hours. There was then a gradual but progressive increase to 52 per cent three days after the injection, that level being maintained to the seventh day. At twenty-four hours the basophil leukocytes had increased to 9 per cent of the total number of leukocytes in the peripheral blood, an average of less than 1 per cent having been present before the injection.

There was no marked change in the number of the Kurloff bodies in the various-sized lymphocytes and monocytes in the smears of the peripheral blood.

GROSS ANATOMIC OBSERVATIONS ON INFECTED GUINEA-PIGS

There was a large amount of peritoneal fluid in the guinea-pigs killed forty-three and forty-eight hours after the injection. The cervical lymph nodes in these animals showed evidence of hemorrhage. The guinea-pigs killed at forty-eight hours had very small mesenteric lymph nodes; in those killed at three days the corresponding nodes were so small that it was difficult to obtain a piece for section. In the animal killed four days after the injection, the mesenteric lymph node was

larger than normal, and increases in size were shown with each successive day, that in the guinea-pig killed seven days after the injection being 3.5 cm. in its longest dimension. In this guinea-pig, in a small portion of the mesentery the lymphatic vessels were swollen and were separated by small white nodules which in sections were found to be developing lymph nodes.

MICROSCOPIC OBSERVATIONS ON INFECTED GUINEA-PIGS

Spleen.—In the spleen of the control uninfected guinea-pig the white pulp appeared as diffuse lymphatic tissue which formed a sheath for the branches of the arteries and contained rather ill-defined lymphatic nodules. Pale-staining central areas were present in some; a few of these were typical reaction centers, with prominent reticular cells, free macrophages and some cellular débris, while others consisted mainly of medium lymphocytes. The sinuses of the red pulp contained a moderate number of small lymphocytes, free macrophages and erythrocytes. There were also some heterophil leukocytes and an occasional monocyte. The nuclei of the reticular cells were prominent in the Billroth cords. The free cells here were mainly small and medium lymphocytes and heterophil leukocytes.

One hour after injection of the infecting dose of Bact. monocytogenes, practically all of the nodules and most of the lymphatic tissue sheaths had more or less well defined margins of medium lymphocytes. Within these margins the predominant free cell was the small lymphocyte. In some of the lymphatic tissue sheaths there were isolated, well demarcated compact accumulations of medium lymphocytes. The red pulp near the margins of the sheaths contained many more medium-sized lymphocytes than were seen in the control spleens. There were increased numbers of heterophil leukocytes in both the sinuses and the cords. The reticular cells throughout the red pulp showed no change and no mitoses.

At three hours, the margins of the lymphatic tissue sheaths, which had been sharply demarcated, were spreading out into the red pulp. Most of the white pulp was less dense than at the one hour stage, and its reticular cells were more prominent. Throughout the lymphatic tissue sheaths there were a number of lymphocytopoietic centers in the nodules. Many of these in turn had reaction centers within them. The cross-section of one lymphatic tissue sheath contained two nodules with lymphocytopoietic centers. Accumulations of plasma cells and lymphocytes were prominent around the trabeculae. The lymphocytes containing Kurloff bodies were increased in number in the sinuses and cords, and the latter contained great numbers of plasma cells. The reticular cells of the cords showed no change.

At six hours, the periarterial lymphatic tissue sheaths in most places were very thin and quite sharply demarcated from the red pulp. The nodules were smaller and contained small lymphocytes centrally; some of the nodules were surrounded by a zone of medium-sized lymphocytes; others, by more densely packed small lymphocytes. There were only a few small areas of active proliferation of lymphocytes in the diffuse lymphatic tissue of the periarterial sheaths.

The sinuses had many more cells than at the previous stage; the heterophil leukocytes were increased in number, and there were a few circulating myelocytes and an increased number of monocytes. The majority of the Kurloff bodies were in medium-sized and small lymphocytes. There were some large basophil lymphocytes in the pulp cords; many of them were ameboid. One reticular cell in the red pulp was in mitosis. A few basophil myelocytes and mature basophil leukocytes were present in the cords.

At nine hours, the lymphatic tissue sheaths were still narrow, and their margins merged gradually into the red pulp. Most of the nodules were larger and were surrounded by zones of medium-sized and small lymphocytes, with an occasional large lymphocyte among them. Some of the arterioles of the red plup were surrounded by zones of medium and small lymphocytes. The number of basophil leukocytes in the red pulp was increased and a few eosinophil leukocytes were present. There were only a few Kurloff bodies in the many lymphocytes of the red pulp. The reticular cells of the red pulp showed no change.

At twelve hours, the sinuses were crowded with lymphocytes of all sizes; some of these were medium-sized lymphocytes, many of which were in mitosis. There was also an increase in the number of large basophil lymphocytes in the cords and sinuses. The lymphatic tissue sheaths were much narrower than in the previous animals, but the nodules were more numerous than at nine hours. From the borders of the sheaths irregular areas of medium lymphocytes extended far into the red pulp; in these areas were many ameboid lymphocytes. Many of the larger nodules had reaction centers; a few had lymphocytopoietic centers. Despite the crowding of the sinuses and pulp cords with lymphocytes, the reticular cells were still distinct, and at this time no mitoses were found in them.

At eighteen hours the periarterial lymphatic tissue was much more extensive than at any previous stage, and its margins extended further into the red pulp. These lymphatic tissue sheaths were wide, and each along practically its entire course had an inner zone of predominantly small lymphocytes and fairly prominent reticular cells and an outer denser zone of medium-sized and larger lymphocytes. This outer zone was not sharply circumscribed and merged into the red pulp. Here and there along the sheaths were many small nodules of proliferating lymphocytes. Some of the sheaths in cross-section were quite large. One had four nodules consisting of proliferating medium-sized lymphocytes, while some sheaths had two or three such areas. Some nodules in the cross-section had an outer zone of medium-sized lymphocytes, while others consisted of densely packed medium-sized lymphocytes, many of which were in mitosis, some larger lymphocytes and a few prominent reticular cells.

Large basophil lymphocytes were very numerous in the red pulp, and many of these were in mitosis. In areas adjacent to the margins of the sheaths it was very easy to see that these large lymphocytes had moved into the red pulp from the lymphatic tissue of the sheaths. There were many ameboid lymphocytes in these regions of the red pulp. There was no evidence of the multiplication of the reticular cells of the red pulp or of their transformation into the numerous medium-sized and large lymphocytes that crowded the red pulp. There were many basophil leukocytes in the sinuses, and the monocytes were more numerous here than at twelve hours. Numerous areas of plasma cells were also to be found throughout the red pulp.

At twenty-four hours, the periarterial lymphatic tissue sheaths were very large. This white pulp extended far into the cords of Billroth. Many of the lymphocytopoietic centers of the nodules had paler-staining central areas of prominent reticular cells and a few macrophages; these were beginning reaction centers. At the same time cross-sections of several of the lymphatic tissue sheaths showed four or five lymphocytopoietic foci. Several of the larger foci were somewhat circumscribed by marginal zones of smaller lymphocytes, but two smaller ones in particular were areas of lymphocyte proliferation beginning in the diffuse lymphatic tissue of the sheath independently of a preexisting nodule.

The predominant free cell in the red pulp was a large basophil lymphocyte. Many of the large basophil lymphocytes in the cords were dividing. The reticular

cells here were quiescent and showed no evidence of proliferation. The sinuses contained many monocytoid cells. There were only a few Kurloff bodies, and the number of plasma cells was markedly increased. The spleen up to this time had reacted to the infection with a marked increase in the number and size of the lymphocytes in the red pulp; these lymphocytes for the most part had migrated here after their extensive multiplication in the lymphatic nodules and, to a somewhat lesser extent, in the diffuse lymphatic tissue of the periarterial sheaths. This formation and migration were so great that in many places the margins of the white pulp had extended far into the red pulp; in such areas only the sinuses distinguished the former red pulp from the white.

At thirty-six hours, there was a distinct change in the cellular content of the sinuses and cords. The majority of the lymphocytes were smaller and less basophil; many of them had the appearance of monocytoid lymphocytes. The large nodules were still numerous in the sheaths, although they were less frequent than at twenty-four hours; there were a very few small ones at this time. The margins of the periarterial lymphatic tissue sheaths and, in places, of their larger nodules extended far out into the red pulp. At this time the outer zone of both the nodular and the diffuse lymphatic tissue consisted mainly of monocytes and monocytoid lymphocytes.

The term "monocytoid lymphocytes" includes intermediate cells which cannot be classified as lymphocytes or as monocytes. This group includes many cells whose nuclei have the characteristic large clumps of chromatin, one or more distinct, fairly acidophil nucleoli and the rather thick nuclear membrane of the lymphocyte. The cytoplasm of these cells is more abundant and in some cases paler-staining than that of the typical small and medium-sized lymphocytes. In other cells the nuclei are somewhat larger and more irregular in outline, and the chromatin particles are less clumped than in the lymphocytes, while the nucleoli are smaller and more numerous.

Many of the centers of the lymphatic nodules were pale-staining because of their conspicuous reticular cells. Often some of these cells were just retracting their cytoplasm and becoming basophil. While many of the centers of the nodules were exhausted and practically devoid of lymphocytes, in others there were a few lymphocytes in mitosis. In some of the nodules there were monocytoid lymphocytes and a few monocytes. These monocytes were easily distinguished from the adjacent lymphocytes by their indented nuclei with one or more acidophil nucleoli. The cytoplasm of these cells was abundant in comparison with the small size of the nucleus and stained a pale blue.

At forty-three hours the relative amount of white pulp, as well as its lymphocytopoietic activity, was considerably less than at thirty-six hours. The periarterial sheaths were almost depleted of larger lymphocytes, the cells being mainly small lymphocytes and numerous monocytoid lymphocytes. The nodules were small and far less numerous than at thirty-six hours. In cross-section the lymphatic sheaths appeared small, for the most part. Several larger sheaths in cross-section had medium and a few large lymphocytes on one side and monocytoid lymphocytes and monocytes on the other. A few of the larger nodules appeared to be almost entirely monocytic.

The peritrabecular areas contained more medium-sized and small lymphocytes and fewer plasma cells than previously. Throughout the cell-crowded sinuses and

pulp cords it was very difficult to classify all of the medium-sized cells as either lymphocytes or monocytes. The sinuses were filled with all transition stages from various-sized lymphocytes to monocytes. The reticular cells of the pulp and the lining cells of the sinuses showed no change. There was no evidence of their transformation into monocytes at this or previous stages.

At forty-eight hours, the periarterial lymphatic tissue sheaths had practically disappeared except for a few scattered large nodules, some of which had centers of proliferating lymphocytes. There were many more monocytes and monocytoid lymphocytes throughout the spleen (fig. 1 *B*). As in all previous stages the reticular cells were quiescent and showed no evidence of change. Groups of plasma cells were again prominent, especially around the trabeculae.

At three days, the cords and sinuses of the red pulp were full of monocytes and monocytoid lymphocytes. Many lymphoid cells in the red pulp were in mitosis. The periarterial lymphatic tissue sheaths were slightly more prominent than at forty-eight hours. But instead of the free cells being mainly lymphocytes as in the normal animal, there were monocytoid lymphocytes and monocytes in great numbers in both the diffuse tissue and the nodules of the lymphatic sheaths. Along these sheaths were scattered areas of lymphocytic proliferation, but even in these areas one could find monocytes. There was no evidence of changes in the reticular cells of the red pulp. There were some very large lymphocytes here and there throughout the section. The foci of plasma cells were few and small.

At three and a half days, the periarterial lymphatic tissue sheaths were considerably more extensive than during the previous twenty-four hours; the margins were not sharply delimited from the red pulp. The nodules were larger and somewhat more numerous than at three days. There was an extensive infiltration of actively ameboid lymphocytes of all sizes into the cords and sinuses of the red pulp adjacent to these spreading margins of the sheaths. The nodules and the diffuse lymphatic tissue of the sheaths contained many large lymphocytes; some of these were in mitosis. The entire white pulp appeared diffusely lymphocytopoietic, although this process in a few places was accentuated in foci of proliferating lymphocytes. There were no mitoses in the reticular cells of the red pulp or evidence of their change into lymphocytes, although many of the lymphocytes in these areas were actively multiplying. The cords and sinuses of the red pulp were crowded with lymphocytes which had migrated into the red pulp from the white pulp. The number of monocytes was still high in the cords and sinuses but had decreased appreciably in the lymphatic tissue of the arterial sheaths. The regions around some of the trabeculae appeared as diffuse lymphatic tissue; plasma cells were very infrequent in these areas.

At four days, the increase in the lymphatic tissue around the arteries was still more marked than at the previous stage. The numerous nodular areas of proliferating lymphocytes throughout it varied greatly in size as well as in structure. Some of these lymphocytopoietic foci were sharply demarcated by smaller lymphocytes; others had no delimiting corona of densely packed cells, while in some there were three or four concentric zones of lymphocytes of different sizes, showing evidence of several different cycles of formation of lymphocytes in one nodule. The cross-section of one sheath showed three centers of proliferating lymphocytes in one lymphatic nodule. The outer zones of many of the nodules contained exceedingly numerous monocytes. The cords and siuses contained many monocytes and monocytoid lymphocytes; the heterophil leukocytes were abundant throughout the red pulp.

At five days, the medium-sized and large lymphocytes were more numerous throughout the entire spleen, but especially in the lymphatic tissue sheaths, than

at any previous hour. The lymphatic nodules in the sheaths were very large and contained many proliferating medium-sized and large lymphocytes. The reticular cells of the red pulp appeared normal. The cords and sinuses still contained many monocytes and all stages of monocytoid lymphocytes. Heterophil leukocytes were still very numerous in the red pulp, and there were a few circulating myelocytes and megakaryocytes.

At six days, the cords and sinuses were crowded with lymphocytes of all sizes, monocytes, monocytoid lymphocytes and many heterophil leukocytes; erythrophagocytosis by macrophages was marked. The reticular cells of the red pulp appeared inactive. Plasma cells were very numerous in both cords and sinuses.

The periarterial lymphatic tissue of the sheaths was extensive, and throughout it were many lymphatic nodules. The margins of some of these were sharp; others had many ameboid cells migrating out from them. In a few nodules paler-staining reaction centers had appeared, while in others the pale-staining central portions consisted mainly of very large lymphocytes. There were only a few monocytes in the white pulp.

At seven days, the sinuses of the red pulp were crowded with small and medium-sized lymphocytes, monocytes and monocytoid lymphocytes. There were some macrophages and heterophil leukocytes and some small lymphocytes with Kurloff bodies. The increase in the lymphatic tissue of the periarterial sheaths was even greater than at six days, and many of the nodules showed three or four different cycles. In these the centrally located medium lymphocytes were surrounded by a ring of small lymphocytes, and these in turn by an external zone of medium-sized and large lymphocytes; while in others the central area was made up of large basophil lymphocytes, with some in mitosis, and around these was a zone of medium lymphocytes, with many in mitosis. The latter in turn were surrounded by one or more zones of small and large lymphocytes.

Summary.—The first reaction in the spleen of the guinea-pig to the injection of Bact. monocytogenes is a movement of lymphocytes from the lymphatic tissue of the periarterial sheaths into the red pulp. This loss of lymphocytes by the lymphatic tissue is quickly compensated by an intensive new formation of lymphocytes in these sheaths. During the first twenty-four hours of the infection more lymphocytes are formed here than are lost. The extensive migration of lymphocytes from the sheaths is the source of the many large and medium lymphocytes that crowd the red pulp at twenty-four hours. These lymphocytes are connected by an intimate series of transition forms (monocytoid lymphocytes) to the many monocytes present in the red pulp. These transitions are more frequent during the second than during the first day. At thirty-six and forty-eight hours and during the third day these monocytoid lymphocytes, as well as recognizable monocytes, are present not only in the red pulp but also in the diffuse and nodular lymphatic tissue of the periarterial sheaths. During this entire time there is no evidence of any change in the reticular cells of the red pulp, and no mitoses are observed in them. The monocytes present in the spleen develop by individual hypertrophy and transformation of the lymphocytes.

Mesenteric Lymph Node.—In the control node most of the nodules were small and were confined mainly to the outer cortical zone, with a few along the corticomedullary junction. Pale-staining centers were present in the majority of them and consisted mainly of medium lymphocytes with a few mitoses. There were a few which had small centers of reticular cells. The diffuse lymphatic tissue of the cortex was a fairly uniform mass of medium-sized and small lymphocytes and a few scattered large basophil lymphocytes, with here and there an inconspicuous reticular cell. The subcapsular sinus was not distended and contained mainly smaller lymphocytes. The cells in the medullary cords and in the sinuses were small and medium-sized lymphocytes, some macrophages and only an occasional heterophil leukocyte.

At one hour after injection of the infecting dose of Bact. monocytogenes, the even distribution of lymphocytes in the diffuse tissue of the cortex, as observed in the control mesenteric lymph node, was lacking. The lymphocytes at the margins of the nodules in the outer cortex were ameboid. There were irregular dense accumulations of medium-sized lymphocytes in the midcortical and corticomedullary regions. There were more macrophages and red blood cells in the sinuses than in the normal node. The nodules showed no change.

At three hours, in many places in the diffuse cortical tissue the reticular cells were prominent, there being few small and medium-sized lymphocytes in these areas. The nodules in the outer cortical zone for the most part had centers of reticular cells, a few free macrophages and very few lymphocytes; only an occasional nodule in this area showed lymphocytes in mitosis. The subcapsular sinus contained many more cells, which were predominantly medium-sized lymphocytes. The corticomedullary region contained more nodules than previously and these varied considerably in their structure. A few of these nodules were small foci of medium-sized lymphocytes without a sharp outer zone of small lymphocytes. These small foci contained varying numbers of mitotic figures. The larger areas of proliferating medium-sized lymphocytes in this region were circumscribed by densely packed small lymphocytes. But all of the nodules in the corticomedullary region were actively lymphocytopoietic. The free macrophages were very prominent in the sinuses throughout the node but especially in the medulla. They contained much waste pigment and cellular débris.

At six hours, the diffuse lymphatic tissue of the cortex was again densely packed with small and medium-sized lymphocytes and some large lymphocytes. The larger nodules in the cortex had large pale-staining centers; in some of these, part of the pale area contained prominent reticular cells, some dead cells and macrophages, while the other portion was filled with closely packed medium-sized and larger lymphocytes, some of which were in mitosis. Others of these pale-staining centers consisted mainly of medium-sized and large lymphocytes, again with many in mitosis, while a few others were reaction centers, i. e., with few lymphocytes, prominent reticular cells, some macrophages and cellular débris, with no central areas of proliferating lymphocytes. There were in addition several very large homogeneous nodular areas made up entirely of medium-sized and large lymphocytes, with many in mitosis. These large lymphocytopoietic nodules were not circumscribed by a zone of small lymphocytes.

Most of the nodules in the corticomedullary region were smaller than those in the rest of the cortex, and nearly all had an outer dark-staining zone of small lymphocytes surrounding a paler-staining central area of medium-sized lymphocytes. In this region, as well as in the diffuse lymphatic tissue of other parts of the cortex described, there were small and large areas of medium-sized lymphocytes with many mitotic figures which were not demarcated from the adjacent

diffuse lymphatic tissue by a zone of small lymphocytes. Lacking this outer zone of small lymphocytes, they may be considered as "bare" lymphocytopoietic nodules in the diffuse lymphatic tissue, in contrast to similar foci which appeared in pre-existing nodules and thus were covered with a layer of small lymphocytes.

Because of the increased lymphocytopoietic activity at this hour, more lymphocytes were produced than left the organ. The sinuses contained large numbers of medium-sized and small lymphocytes and a few free macrophages filled with cellular débris.

At nine hours, the number of larger lymphocytes in the diffuse lymphatic tissue of the cortex was increased. The numerous nodules in the outer cortical zone were large, and most of them consisted chiefly of medium-sized and very large lymphocytes; mitoses in the lymphocytes were numerous. Most of these nodules had no outer limiting zone of small lymphocytes; a few of the larger ones had outer dark-staining zones of small lymphocytes and pale-staining central areas consisting of reticular cells, macrophages, a few lymphocytes and cellular débris; these were "reaction centers." In a few of them there were eccentric areas of medium-sized lymphocytes, some of which were in mitosis. A further variation in nodular structure was the appearance of an eccentric pale-staining zone of proliferating medium-sized lymphocytes in a nodule consisting elsewhere only of small lymphocytes. Again, as at six hours, rounded dense areas of proliferating medium-sized lymphocytes, which bore no relation to a preexisting nodule, could be found in both cortex and medulla.

The medullary cords and sinuses were so crowded with medium-sized and small lymphocytes that the normal architecture was obscured. Many of the medium lymphocytes in the medullary sinuses were in mitosis. There were many free macrophages throughout the node, and eosinophil leukocytes were numerous. Again, as in the one-hour node, there were irregular dense accumulations of ameboid medium-sized and small lymphocytes infiltrating the midcortical area; they showed no evidence of multiplication in this place.

At twelve hours, reaction centers of the type described were beginning to appear in the larger nodules in the cortex. The number of mitoses in the nodules was less than at the previous stage, and reticular cells were more prominent in all of them. Only a few smaller nodules had central areas of medium-sized and larger lymphocytes. In the diffuse tissue of the cortex the cellular stroma was quite prominent; the predominant cells were the large lymphocytes, and these were also numerous in the medulla. The subcapsular sinus had many medium-sized and small lymphocytes as well as monocytoid lymphocytes with small lymphocyte nuclei and a considerably increased amount of cytoplasm. Mitoses were frequent in both medium-sized and large lymphocytes in the medullary sinuses; here plasma cells were also numerous.

At eighteen hours, the lymph node was smaller than at earlier stages, and its cortex was narrow. The nodules in the cortex were numerous but small, and most of them consisted of large outer zones of small lymphocytes with lighter-staining central areas. In all of these pale centers the medium-sized and large lymphocytes were the predominant cells, although the ratio between the two sizes differed in each nodule. In some nodules there were few "tingible bodies" and an occasional macrophage. A few of the larger nodules in the cortex were compact masses of medium-sized and large lymphocytes not demarcated by layers of small lymphocytes from the diffuse lymphatic tissue.

The diffuse lymphatic tissue of the cortex was fairly well filled with small and medium-sized lymphocytes, but in the medulla there were few lymphocytes of any size in either the cords or the sinuses (especially the latter). The reticular

cells of the medulla were prominent. There were several irregular dense accumulations of smaller lymphocytes in the corticomedullary region, similar to those described nine hours after the injection. Plasma cells were numerous in the medullary cords, and there were a few free macrophages in the sinuses.

At twenty-four hours, in sharp contrast to the previous stage, the mesenteric lymph node was large and had many nodules in both cortex and medulla. These varied in size from small isolated areas of proliferating medium-sized lymphocytes in the medulla to the very large nodules in the cortex. Many of the large nodules, with pale-staining central areas consisting of medium-sized and large lymphocytes with many mitotic figures, had dense peripheral zones of smaller lymphocytes, while other large areas of proliferating lymphocytes lacked this dark-staining outer zone. The number of dividing lymphocytes within the nodules was far greater than the number in the diffuse lymphatic tissue outside of the nodules in both cortex and medulla. The medullary cords and sinuses were again crowded with lymphocytes of all sizes and free macrophages. In the sinuses a few large free macrophages with débris-laden cytoplasm were in mitosis. Here there were also a number of monocytes and atypical cells which could not be classified as either monocytes or lymphocytes. Most of these had more cytoplasm than lymphocytes, although they still had lymphocytic nuclei, while in others with less cytoplasm, the nuclear pattern was changed in that the chromatin particles were in smaller clumps along the thinner nuclear membrane. These were classified as monocytoid lymphocytes (fig. 1 *A*). These cells had not been present in the lymphatic nodules but had been increasing in number in the subcapsular sinus, which contained some recognizable monocytes as well as many of these monocytoid lymphocytes.

At thirty-six hours, the corticomedullary junction contained several large, irregular, dense accumulations of small and medium-sized lymphocytes. The smaller lymphoctes were the predominant cells in the diffuse cortical tissue. Pale-staining areas of reticular cells were present in some of the lymphatic nodules, but for the most part the nodules were smaller and lymphocytopoietic. Small areas of proliferating medium-sized lymphocytes with or without peripheral zones of small lymphocytes were scattered through the medulla. The subcapsular sinus contained many lymphocytes, mainly of the smaller type, a few monocytes and many monocytoid lymphocytes. The medullary cords were crowded with lymphocytes, and the sinuses had many monocytoid lymphocytes. Lymphocytes of all sizes showed these monocytoid characteristics. The sinuses were full of red blood cells, but there did not seem to be any appreciable erythrophagocytosis by macrophages. Most of the macrophages were free from pigment and cellular débris.

At forty-three hours, in a few areas of the cortex the reticular cells were prominent. There were few small lymphocytes here, but there were many macrophages and large lymphocytes in this region. In some cortical areas there were many large lymphocytes and some intermediate forms between these and reticular cells, while in other portions of the cortex were dense masses of small and medium-sized lymphocytes. The nodules of the cortex were, for the most part, large and had lymphocytopoietic centers; there were a few smaller foci of proliferating lymphocytes not demarcated from the surrounding diffuse lymphatic tissue.

The medulla had several very large, more or less circumscribed nodules, made up mainly of medium-sized lymphocytes with many mitoses. The medullary cords were filled with larger lymphocytes, while those in the sinuses were smaller. Here also were many macrophages, some monocytes and a few monocytoid lymphocytes.

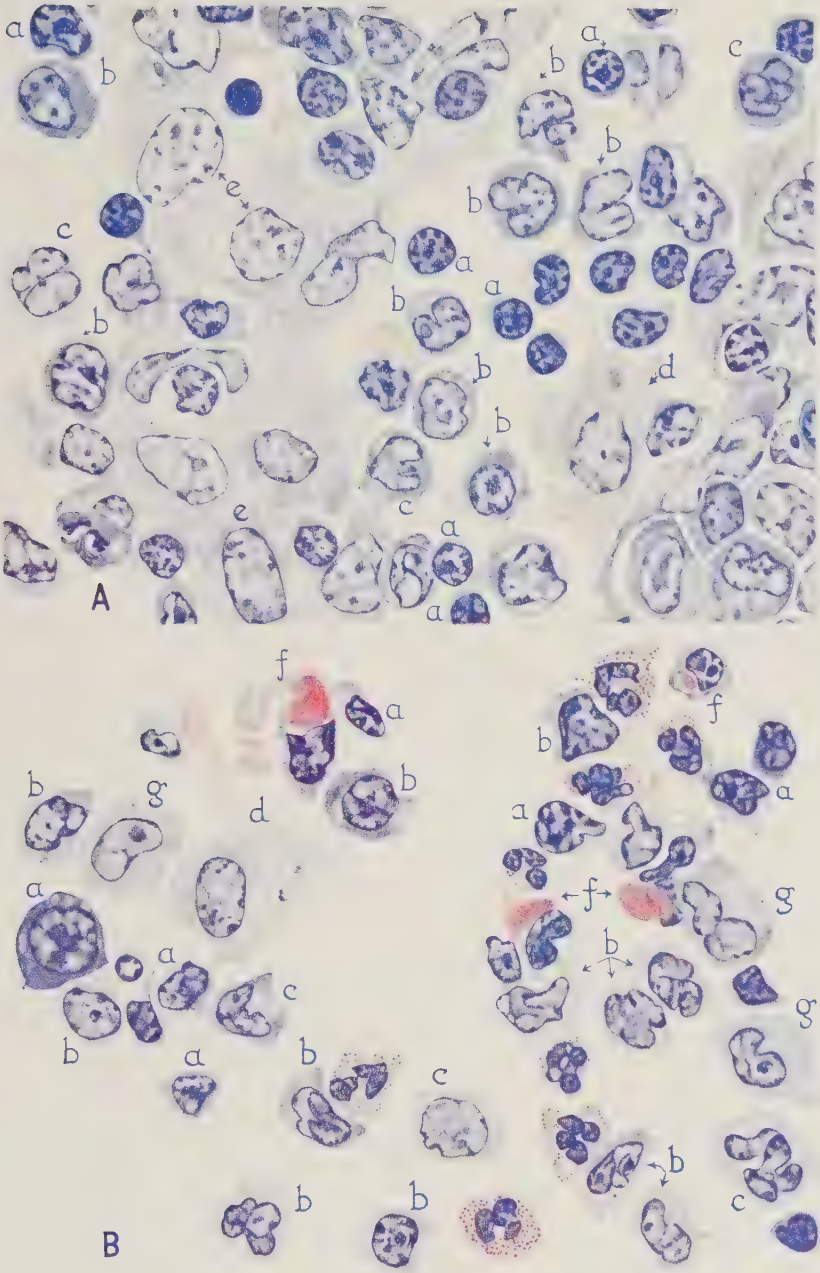
The subcapsular sinus had many monocytes, and there were whole groups of small lymphoid cells with increased amounts of cytoplasm whose nuclei showed rather marked indentations and a dispersion of the larger chromatin particles. In others of these the nucleoli were less prominent. Accompanying these groups of monocytoid lymphocytes were numerous typical small lymphocytes. These monocytoid groups contained all transitions from typical small lymphocytes to obvious monocytes.

At forty-eight hours, the stroma throughout the greater part of the cortex was very prominent, and the number of lymphocytes here was markedly decreased. The margins of most of the nodules in the cortex were sharply demarcated by a reticular stroma. Some of the nodules consisted mainly of medium-sized lymphocytes, with some in mitosis, while others had proliferating medium-sized lymphocytes together with macrophages containing pigment and red blood cells. There was no evidence of reticular cells of the cortex outside the nodules transforming into the many large lymphocytes present throughout the node. In the medulla there were numerous foci of proliferating medium-sized and large lymphocytes. Some of these areas had outer zones of smaller lymphocytes, while others were either cricumscribed by the reticulum of the cords or were not demarcated from the surrounding tissue. The medullary sinuses and the subcapsular sinus contained many erythrocytes, while the number of monocytes and monocytoid lymphocytes, especially in the subcapsular sinus, was less than at forty-three hours.

At three days, the lymph node was extremely small, and the cortex contained only a few nodules. These had outer zones of small lymphocytes with central areas of medium-sized and small lymphocytes. There were no monocytes here and only a few in the subcapsular sinus. A few of the medium-sized lymphocytes in the nodules contained Kurloff bodies. The diffuse lymphatic tissue of the cortex was very loose. The free cells were smaller lymphocytes and many monocytoid lymphocytes. The cortical sinuses contained mainly smaller lymphocytes, while those of the medulla had, in addition, large numbers of macrophages.

At three and a half days, the reticular stroma was greatly thickened. This was due to a decrease in the size of the node. It was obvious that this was not the result of hyperplasia of the reticular cells but of a prominence of the reticular cells due to the depletion of free cells in the meshes. There were only a few very large nodules in the outer cortical zone, with eccentric areas of lymphocytic proliferation. The majority of the few nodules present in the node at this time were in the medulla or the corticomedullary region. There were many very large lymphocytes in the diffuse cortical tissue. Some of the reticular cells here had large acidophil nucleoli, while others had basophil cytoplasm. In these areas there were all stages in the transformation of the reticular cells of the diffuse lymphatic tissue into large lymphocytes. There was an occasional reticular cell in mitosis, but the greater majority of the dividing cells were lymphocytes. Nearly all of these mitoses were extranodular.

It is significant that at this time, during the height of the monocytosis in the peripheral blood, many of the reticular cells throughout the diffuse lymphatic tissue were transforming into large and medium-sized lymphocytes. This extensive transformation of reticular cells into lymphocytes occurred only after exhaustive lymphocytopoiesis in the lymphatic nodules. Up to this time the intense lymphocytopoiesis by proliferation of lymphocytes had sufficed to produce enough lymphocytes to meet the



EXPLANATION OF FIGURE 1

A, camera lucida drawing of a medullary sinus of a mesenteric lymph node of a guinea-pig killed twenty-four hours after an injection of Bact. monocytogenes.

B, camera lucida drawing of a venous sinus of the spleen of a guinea-pig killed forty-eight hours after an injection of Bact. monocytogenes.

In *A* and *B*, *a* indicates lymphocytes; *b*, monocytoid lymphocytes; *c*, monocytes; *d*, macrophages; *e*, reticular cells; *f*, Kurloff bodies; *g*, *monocyte→macrophage*. Hematoxylin-eosin-azure II; × 2,240, reduced to 1,490.

need for cells of this type, but the depletion of lymphocytes from the diffuse and nodular lymphatic tissue was now so pronounced that the reserve was gone, the normal mode of lymphocyte production was insufficent and the reticular cells were activated. That they were transforming into lymphocytes and not into monocytes was shown by the finding of all stages intermediate between reticular cells and lymphocytes. In addition, throughout the cortex all stages of transition forms between these lymphocytes and monocytes were present.

There were irregular dense accumulations of medium-sized and small lymphocytes in the inner cortical zone. The medullary sinuses contained many small lymphocytes and macrophages, but the cords were crowded with lymphocytes. The reticular cells were prominent here, and occasionally one could be found in division. There were several heterophil myelocytes in the medullary sinus. At this time the small lymphocytes and monocytes in the subcapsular sinus were connected by a complete series of transition forms.

At four days, the lymph node was somewhat larger, and the reticular cells of the cortex were much less prominent than at three and a half days because of an increase in the number of lymphocytes. Most of these had developed from the reticular cells, which were described in the preceding stage as developing into lymphocytes. There were few lymphocytopoietic nodules in the cortex. The medulla had a few smaller foci of lymphocyte proliferation, and the lymphocytes at the margins of these were ameboid. The medullary sinuses and cords had many smaller lymphocytes.

At five days, the lymph node was larger than at four days. In a few localities in the cortex the reticular cell stroma was again prominent, but the greater part of the diffuse tissue was crowded with lymphocytes of all sizes; here the reticular cells were inconspicuous. Throughout the cortex and medulla were numerous nodules which varied greatly in structure and in size. A few larger ones consisted entirely of proliferating medium-sized lymphocytes; others had centers of dividing medium-sized and large lymphocytes, while a few contained reaction centers. Throughout the cortex were many small nodular areas of medium-sized lymphocytes, many of which were in mitosis. These appeared to be new lymphocytopoietic nodules forming in the diffuse lymphatic tissue. The sinuses and medullary cords had many macrophages filled with a débris of red and white blood cells. There were some monocytes here and in the subcapsular sinus.

At six days, the sinuses throughout the node, as well as the diffuse lymphatic tissue, were more densely crowded with lymphocytes than at five days, but the number of nodules in the outer cortical area was not as great. In the corticomedullary region and in the medulla the nodules were large and consisted mainly of medium-sized lymphocytes, many of which were in mitosis. In the lymphocytopoietic centers within several nodules in the cortex there were a few early heterophil myelocytes arising from medium-sized lymphocytes.

At seven days, the mesenteric lymph node was larger than at any previous time during the infection or in the controls. The diffuse lymphatic tissue of the cortex was densely packed with medium-sized and small lymphocytes and many larger lymphocytes, and the cortical sinuses were crowded with small lymphocytes. The cortex had become progressively larger since the fourth day. The nodules were numerous throughout the cortex and the medulla. Some of these in the cortex

had several cycles of lymphocytopoiesis, i. e., a central light-staining zone of medium lymphocytes surrounded by smaller ones, and this in turn had one or more peripheral zones of larger lymphocytes.

Some of the nodules had medium-sized lymphocytes, some of these in mitosis, and in addition these areas contained varying numbers of monocytes. The subcapsular sinus also contained monocytes, monocytoid lymphocytes and lymphocytes. Some of the smaller nodules in the medulla had pale central zones of reticular cells and macrophages with darker peripheral zones of small lymphocytes, but the majority of the nodules in the medulla consisted mainly of medium-sized lymphocytes, with many in mitosis. Some of these areas of lymphocyte proliferation had limiting peripheral zones of smaller lymphocytes, while many other areas lacked this zone. The medullary sinuses contained macrophages, many lymphocytes and some monocytes.

Summary.—The mesenteric lymph node of the guinea-pig, like the spleen, responds to the injection of Bact. monocytogenes with a movement of lymphocytes from the diffuse lymphatic tissue. Nine hours after the injection this initial depletion is compensated by extensive new formation of lymphocytes. At the end of the first day and during the entire second day these reserve and newly formed lymphocytes are connected with typical monocytes by an intimate series of transition forms. By the end of the second day the depletion of lymphocytes is so extensive that the new formation of these cells does not keep pace with it, and the cortex becomes thin and the node small. This decrease in size becomes progressively greater during the next twelve hours so that on the third day the lymphatic nodules are exhausted of lymphocytes. But even at this time there is no evidence of any cells other than lymphocytes being the source of the many monocytes present throughout the node.

During the succeeding four and a half days there is progressive recovery from the depletion present three days after the injection. This takes place first by individual transformation of reticular cells into lymphocytes as found in the guinea-pig killed three and a half days after infection. During the fourth day many new lymphatic nodules appear, both in the cortex and in the medulla. By seven days the lymph node is more than twice the size of that in the control animal; the cortex and, to a lesser extent, the medulla are crowded with lymphocytes of all sizes. Lymphocytopoiesis is very extensive in the many nodules throughout the node.

Cervical Lymph Node.—In the first twelve hours after the injection of Bact. monocytogenes the cervical lymph nodes showed little or no change from those of the control guinea-pigs. There was an occasional monocyte in the medullary sinuses and a few heterophil leukocytes; cells of these types were also found in the control animals. During the next twelve hours the number of monocytes in the sinuses increased appreciably; a few of them contained Kurloff bodies.

At thirty-six hours large groups of plasma cells and of myelocytes were present in the inner cortical zone and in the medullary cords; erythrophagocytosis

by the macrophages of the sinuses was marked. Monocytes were present throughout the medullary portions of the node. There were many medium-sized lymphocytes in mitosis in the lymphatic nodules, but the cervical lymph nodes did not show the cyclic changes of the mesenteric group. The depletion of lymphocytes from the cortex of the node was not nearly as marked here as in the mesenteric node.

In the animals killed five days after the injection (at the time when the mesenteric lymph nodes were increased in size) the cortex of the cervical node was also greatly increased. There were many more lymphocytopoietic nodules than previously. The medullary cords were distended, and the sinuses were crowded with cells. Among these, the medium-sized and large basophil lymphocytes were prominent, and both types showed many mitoses. The large free macrophages were filled with engulfed erythrocytes. Heterophil and eosinophil myelocytopoiesis was marked in the diffuse lymphatic tissue and occasionally was found in the nodules. Most of the early myelocytes of these two groups had lymphocytic nuclei.

The cervical lymph node on the sixth day after the injection showed extensive myelocytopoiesis, both from lymphocytes and from the reticular cells; also some megakaryocytes were present in the medulla.

OBSERVATIONS ON THE PERIPHERAL BLOOD OF INFECTED RABBITS

The blood serum of the rabbits, taken before the injection, showed no agglutination in dilutions from 1:10 through 1:5,120 with either the avirulent stock strain of Bact. monocytogenes or with the strain virulent for rabbits.

The average white blood cell count before the injection was 12,500 cells per cubic millimeter. Fluctuations in the total white cell counts similar to those noted for the guinea-pigs during the first twelve hours also occurred in the rabbits. Leukopenia was present also from eighteen to thirty-six hours after the injection, at which time the white cell count was 6,300. Following this the count increased to 19,000 at forty-eight hours and maintained that level at sixty-six hours.

The heterophil leukocytes and the lymphocytes showed approximately the same variations in number as those found in the guinea-pig.

The average percentage of monocytes in the animals before the injection was 3 per cent. The three and the six hour rabbits had no monocytes in the peripheral blood. From nine to thirty-six hours after the injection the monocytes numbered about 6 per cent of the total number of leukocytes. At forty-eight hours they increased to 26 per cent and at sixty-six hours, at the time the number of lymphocytes was low (15 per cent), they increased to 54 per cent of the total number of leukocytes. The basophil leukocyte count constituted 9 per cent of the total number of leukocytes twenty-four hours after the injection.

The heterophil leukocytes in the peripheral blood of the rabbits killed at forty-eight and sixty-six hours after the injection had many toxic granules; some of the monocytes at these same times contained phagocytosed erythrocytes, and the cytoplasm of others was markedly vacuolated. The red blood cells showed no apparent change during the first twenty-four hours following the injection, but at thirty-six hours poikilocytosis was apparent, and some normoblasts were present. At forty-eight hours there was a marked increase in the number of normoblasts; polychromatophilia was pronounced; there was marked basophilic stippling, and anisocytosis and poikilocytosis were increased in degree as well as in frequency.

GROSS ANATOMIC OBSERVATIONS ON INFECTED RABBITS

During the first twenty-four hours after the injection of Bact. monocytogenes there was a marked increase in the size of the mesenteric lymph node. A decrease in size was apparent at thirty-six hours, and this decrease continued until at sixty-six hours the mesenteric lymph node was so small that an accurate measurement could not be obtained. The brown color which it presented grossly was the normal color of the medulla.

The spleen was also increased in size, but, unlike the mesenteric lymph node, it was even larger during the second day than the first. At sixty-six hours it was blue-red but was not as swollen as the spleen of the forty-eight hour rabbit.

The bone marrow of the femur at thirty-six hours was dark red and quite adherent to the endosteum, while at forty-eight hours it was adhesive along the entire length of the marrow cavity and showed several small gray areas. This adhesiveness was not apparent at sixty-six hours.

MICROSCOPIC OBSERVATIONS ON INFECTED RABBITS

Spleen.—In the spleen of the normal rabbit there was a fairly sharp demarcation between the white pulp and the red. In cross-section the periarterial sheaths were comparatively sharply circumscribed, and the lymphatic nodules within them were for the most part pale-staining, and their centers consisted mainly of reticular cells. The cords and sinuses were distinct, and most of the cells here were small and medium-sized lymphocytes with an occasional monocyte and some heterophil leukocytes.

The spleen of the rabbit studied during the early stages of infection with Bact. monocytogenes up to the time of the appearance of great numbers of monocytes in the peripheral blood showed an initial response to the infection essentially the same as that described for the spleen of the guinea-pig. The rapid early and continued migration of lymphocytes from the white pulp into the red was accompanied by an extensive new production of lymphocytes in the periarterial nodular lymphatic tissue. The latter process did not begin until twelve hours after the injection in the guinea-pig and reached its height in twenty-four hours, compared with eighteen hours in the rabbit. The continued migration of lymphocytes preexisting in the white pulp, as well as of those newly formed, was the source of the many lymphocytes found in the cords and sinuses of the red pulp during the second day. As described for the guinea-pig, at this time there was individual transformation of lymphocytes into monocytes; forms representative of all stages of this transition (monocytoid lymphocytes) were present in the red pulp. There were also many monocytes in the nodules of the white pulp (fig. 2). Up to this time there was no evidence of changes in the reticular cells of the red pulp.

Unlike the guinea-pig, the rabbit at forty-eight hours after the injection showed the lymphatic tissue of the periarterial sheaths more extensive than at thirty-six hours, and many nodules which were lymphocytopoietic. Throughout the diffuse lymphatic tissue of the sheaths were numerous large lymphocytes; many of these were developing from reticular cells. This process also appeared to be taking place in the red pulp but to a far smaller degree. Many of the reticular cells of the red pulp were very prominent, and several were in mitosis. This may have indicated that, though the lymphocytopoiesis of the previous thirty-six hours had been intensive, the lymphocytes preexisting in the lymphatic tissue could not produce sufficient lymphocytes to meet the demand and the reticular

cells of the red pulp were activated. But the greatest amount of lymphocyte production was still confined to the white pulp as evidenced by the greater number of mitoses in the latter.

Sixty-six hours after the injection the nodules, as well as all of the periarterial sheaths in the spleen, appeared depleted of lymphocytes, and their margins consisted mainly of monocytoid lymphocytes and monocytes. In one large nodule the central zone had many macrophages, dead lymphocytes and epithelioid reticular

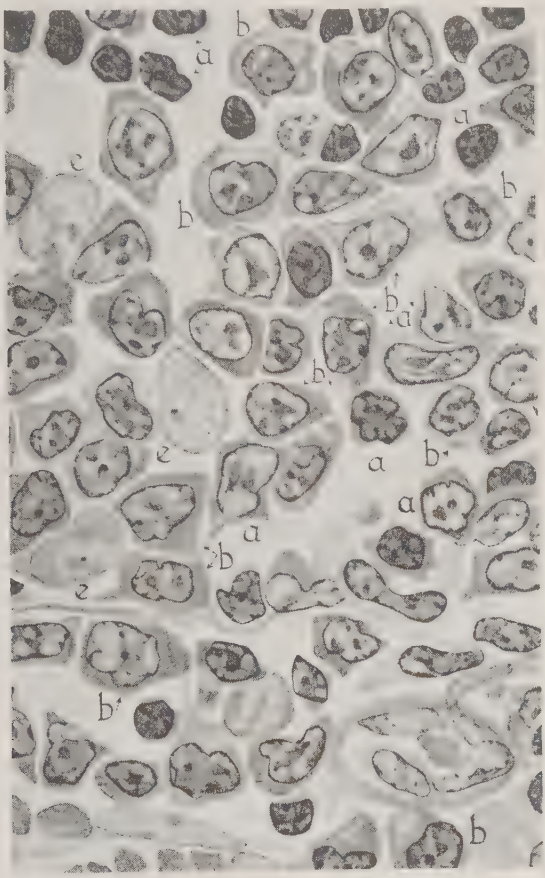


Fig. 2.—Group of monocytoid lymphocytes at the edge of the periarterial lymphatic tissue of the spleen of a rabbit killed forty-eight hours after an injection of Bact. monocytogenes. The small letters and the optical system have the same explanation as in figure 1.

cells, as well as large lymphocytes, with many cells intermediate between these and the reticular cells. In addition, the entire nodule was infiltrated with heterophil leukocytes, many of which were dying.

Throughout the red pulp were numerous islands of heterophil myelocytes. Many of them were free and had nuclei like those of the reticular cells, while some were still attached. In a few areas there were groups of developing

megakaryocytes. The lining cells of the sinuses in the red pulp were actively phagocytic; they contained red blood cells and fragments of dead leukocytes.

More reticular cells of the cords in the red pulp were in mitosis than at forty-eight hours. There were numerous intermediate stages in the formation of large lymphocytes from the reticular cells of the Billroth cords.

It should be mentioned that this is the stage in the infection at which most of the previous workers began to study the organs of their infected rabbits, for at this time the number of monocytes in the peripheral blood is greatly increased. It is obvious that if one started to examine the spleen at this time, when the entire red pulp contains numerous monocytes, the reticular cell proliferation present at this time might give the erroneous conclusion that the monocytes have their origin in the reticular cells (reticuloendothelial). But the series of events leading up to this hour demonstrate, without doubt, that this activation of reticular cells takes place only after exhaustive lymphocytopoietic activity on the part of the lymphocytes in the diffuse and nodular lymphatic tissue of the periarterial sheaths. The monocytes which are present in the spleen in great numbers at this and slightly earlier stages are connected by an intimate series of transition forms with the many lymphocytes filling the red pulp following migration into it from the sheaths. Even at this time the reticular cells of the Billroth cords are developing, not into monocytes, but into large lymphocytes. This is evident from the many transition stages between these two cell types which are present, and in addition throughout the red pulp there are still many large and medium-sized lymphocytes which are becoming monocytoid.

Mesenteric Lymph Node.—In the control the diffuse lymphatic tissue of the cortex was compactly filled with small and medium-sized lymphocytes. Lymphatic nodules were numerous in the outer cortical zone, and most of these consisted of more or less densely packed small lymphocytes (fig. 3 *A*). Only an occasional nodule here had a pale-staining central area of either reticular cells or medium-sized lymphocytes. In the corticomedullary region and in parts of the medulla there were a few of these large nodules, but most of the nodules here were smaller and contained more proliferating medium-sized lymphocytes in their paler-staining centers. The medullary cords and sinuses had only a moderate number of smaller lymphocytes and some macrophages. Eosinophil leukocytes were present here and there throughout the node.

The initial reaction of the mesenteric lymph node of the rabbit to Bact. monocytogenes was essentially the same cytologically as in the guinea-pig; there were mobilization of existing lymphocytes and migration of these cells from the cortex, crowding the sinuses and medullary cords. This migration was accompanied by intensive new formation of lymphocytes, as shown by the great increase in numbers of lymphatic nodules in which numerous mitoses of lymphocytes were found (fig. 3 *B*). There were few or no mitoses in the diffuse lymphatic tissues. During the first twenty-four hours more lymphocytes were produced than left the node, and it accordingly increased in size. During the second day the node decreased in size, most of the nodules in the cortex were depleted of lymphocytes, and many reaction centers appeared (fig. 3 *C*). This lymphocytopoiesis in the

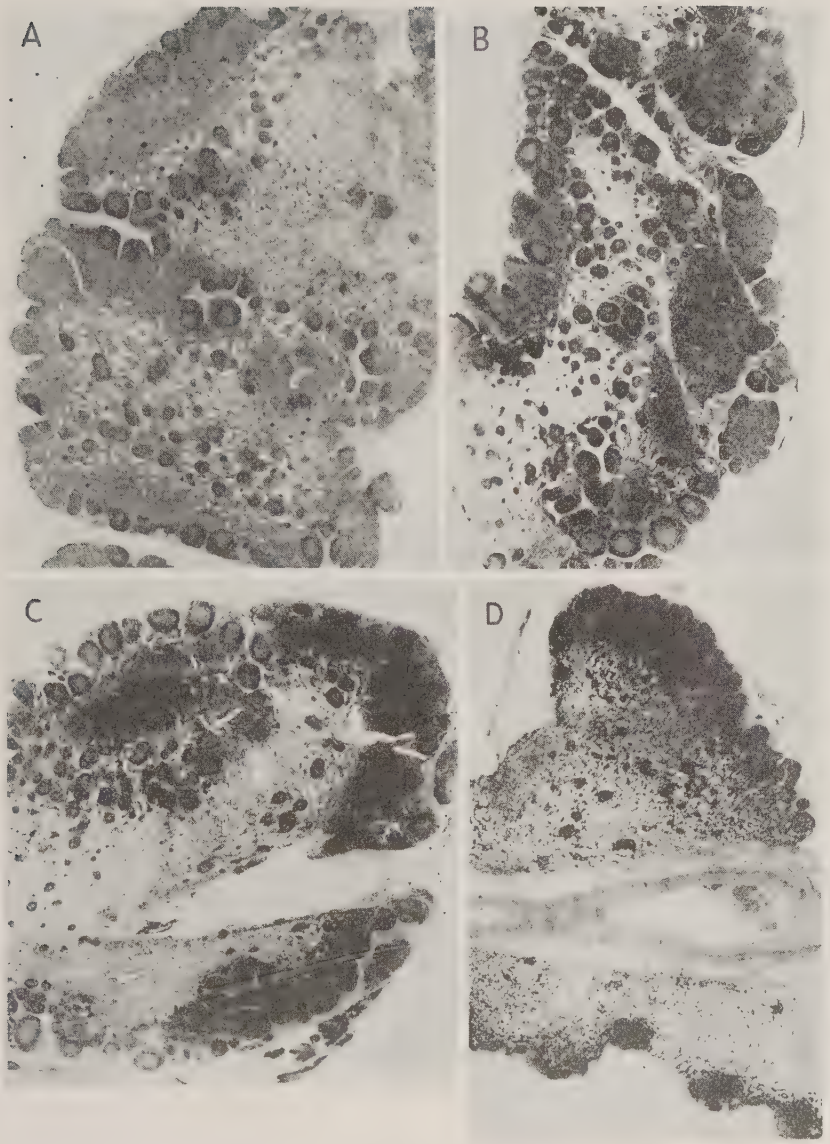


Fig. 3.—Photomicrographs of mesenteric lymph nodes of rabbits after an injection of Bact. monocytogenes. *A,* control, an uninfected rabbit; *B,* infected rabbit killed eighteen hours after the injection; *C,* infected rabbit killed thirty-six hours after the injection; *D,* infected rabbit killed sixty-six hours after the injection. Note the disappearance of the diffuse lymphatic tissue of the cortex and of the nodules in *D*. Here loose lymphatic tissue predominates and often reaches the subcapsular sinus. Hematoxylin-eosin-azure II; × 12, reduced to × 8.

nodules did not produce sufficient numbers of lymphocytes; the cortex became so depleted of lymphocytes that by the third day little diffuse lymphatic tissue remained and most of the node was composed of loose lymphatic tissue resembling that normally found in the medulla (fig. 3 *D*).

At the end of the first day and during the second day before the height of monocytosis in the peripheral blood, there were many transition stages between lymphocytes and monocytes throughout the node. During these early stages of the infection, as well as on the third day, there was no evidence that any cells other than lymphocytes were the source of the many monocytes found in the lymph node thirty-six and forty-eight hours after the injection of Bact. monocytogenes.

Cervical Lymph Nodes.—The cervical lymph nodes of the rabbit did not show the cyclic changes described for the mesenteric group. The depletion of lymphocytes from these nodes was not pronounced nor was there extensive new formation of lymphocytes as in the mesenteric group. But an increase in the number of monocytes began at eighteen hours after the injection of Bact. monocytogenes and became progressively more extensive through the second day. During this time all transitions between lymphocytes and monocytes were present throughout the nodes.

COMMENT

The diverging and at times diametrically opposed results in previously reported studies to determine the source of the monocytosis characteristically caused by Bact. monocytogenes are due in great part to the failure to make cytologic studies of the organs of the infected rabbits at close intervals shortly after the injection of the bacteria. In my experiments it has been clearly demonstrated that during the forty-eight hours previous to the height of the monocytosis of the blood evoked by Bact. monocytogenes the organism produces great numbers of monocytes through individual transformation of the lymphocytes present in the cortex of the mesenteric lymph node and in the periarterial lymphatic tissue of the spleen.

The first stage in the monocytopoiesis is a widespread migration of lymphocytes from these areas of lymphatic tissue during the first nine hours after the injection, which is quickly compensated by greatly increased new formation of lymphocytes. In the guinea-pig this is considerably more marked and appears earlier in the mesenteric lymph node than in the spleen, while in the rabbit there is little difference in time between the reactions in the two organs. This great new formation of lymphocytes, especially in the spleen, is the source of the vast numbers of large lymphocytes crowding the cords and sinuses of this organ about twenty-four hours after the injection.

As early as forty hours before the height of the monocytosis in the peripheral blood is reached, there are many groups of lymphoid cells, particularly in the spleen but also in the mesenteric lymph node, which have more cytoplasm than the smaller lymphocytes. As contrasted with typical lymphocytes, these cells show progressive increases in the inden-

tation of the nucleus, the chromatin particles are more finely divided, and the nucleoli are smaller and more numerous. These changes in the nucleus as well as in the nucleus-cytoplasmic relationship are found in such varying degrees in so many lymphocytes that they constitute an intimate series of transitions from lymphocytes to monocytes. The presence of these transition stages is of particular significance when they are considered in relationship to the time of their appearance. In the early stages of the infection, lymphocytes predominate; between eighteen and twenty-four hours after the injection the first stages of the transition forms appear. At this time only a few monocytes are present. As the process extends, more monocytoid lymphocytes and typical monocytes are present and the number of lymphocytes decreases sharply.

These "monocytoid lymphocytes" are identical with those described by Bloom [2] in the sinuses of the spleen and liver of the rabbit during the height of infection with Bact. monocytogenes. The lymphocyte nature of these morphologic lymphocytes cannot be denied simply because they give rise to monocytes. To do this one would have to postulate that either something had happened to the free cells of the lymphatic tissue and that "monoblasts" had suddenly replaced all of the lymphocytes, or that the "monoblasts" are morphologically identical with the adjacent lymphocytes of the lymphatic tissue. Throughout the initial stages of this infection there are many mitoses in lymphocytes but none in reticular cells. During this time there is no evidence of the rounding up of these fixed cells in either the spleen or the lymph node.

Just previous to the peak of the monocytosis in the peripheral blood, many of the lymphatic nodules in the spleen and in the mesenteric lymph node of both the rabbit and the guinea-pig contain monocytes and many monocytoid lymphocytes as well as typical lymphocytes. In some of these nodules the monocytoid lymphocytes and monocytes are confined to the peripheral part of the nodule and the central part contains mainly proliferating medium-sized lymphocytes; in others they are found throughout the nodule.

At the end of the second day and during the third day after the injection of Bact. monocytogenes, in both the rabbit and the guinea-pig, when the monocytosis in the peripheral blood is well developed and near its height, the first evidences of the transformation of reticular cells into cells of any other type are found in the diffuse lymphatic tissue of the cortex of the mesenteric lymph node and of the periarterial sheaths of the spleen. This occurs after the lymphatic tissue is depleted of lymphocytes in spite of the extensive lymphocytopoiesis of the first forty-three hours after the injection of the bacterium. The reticular cells are prominent now because of the depletion of lymphocytes which have left the tissue and become monocytes. That the reticular cells in these areas are transforming into large lymphocytes and not into monocytes is

clearly shown by the presence of numerous intermediate forms between reticular cells and large lymphocytes; in addition, there are all transitions between these lymphocytes and monocytes in the lymphatic tissue. If the early stages leading up to this one had not been studied, these findings at forty-eight hours would give the impression of a reticular cell hyperplasia. Although the lymphocytopoiesis in the lymphatic nodules of both these organs has been intense, the lymphocytes do not produce sufficient new lymphocytes by mitosis. As a result, the reticular cells are now taking an active part in the production of new lymphocytes in the diffuse lymphatic tissue.

In addition, in the rabbit, beginning at forty-eight hours and proceeding more extensively sixty-six hours after the injection, there are numbers of mitoses in the reticular cells of the red pulp of the spleen as well as all stages of transition between these reticular cells and large lymphocytes. Here again, as in the white pulp, if the study of the spleen had been started at this time, the obvious conclusion might be that the reticular cells were the main source of the monocytes, whereas the findings in the spleen leading up to this stage show that the activation of reticular cells in the red pulp takes place only after intense but insufficient lymphocytopoietic activity on the part of the lymphocytes of the white pulp. Even at this time, which is at the height of the monocytosis in the peripheral blood, the reticular cells of the red pulp are also producing lymphocytes and many of the large and medium-sized lymphocytes in the red pulp are becoming monocytoid. That the reticular cells are not transforming into monocytes or monocytoid cells directly is shown by the finding of a great number of cells intermediate between reticular cells and large and medium-sized lymphocytes, as well as by the fact that the monocytoid cells have predominantly lymphocytic nuclei. They do not have the vesicular nucleus of the reticular cell. On a theoretical basis one could postulate the origin of the monocyte from the fixed reticular cell of either phagocytic or undifferentiated nature. But in my material evidence for this transformation is completely lacking in all of the animals examined. Nor do I find any evidence to support the claims that the hemohistioblast of Ferrata or a free reticular cell is the stem cell of the monocyte (Rinehart;[9] Rezzesi[5]). There is no evidence of a transformation of the reticulo-endothelial cells of the spleen, liver and lymph node into these free cells nor of the transformation of the fixed cells directly into "monoblasts" other than lymphocytes.

One of the few points on which most of the previous investigators of the origin of the monocyte agree is the conclusion that the mesenteric lymph node is not involved in the monocytic reaction in either the normal rabbit or that infected with Bact. monocytogenes. Cunningham, Sabin

9. Rinehart, J. F.: Arch. Path. **13**:889, 1932.

and Doan [10] were unable to find any monocytes in the supravital smears of the mesenteric lymph node of the rabbit. Forkner,[11] using his supravital technic, examined many of the various lymph nodes of rabbits, guinea-pigs and rats and concluded that normally monocytes develop constantly in large numbers in all of the lymph nodes of the body except the large mesenteric group. He claims that his monoblast is easily demonstrated in the paraffin section of the supravitally stained peripheral lymph node "by the fact that it is in close association with premonocytes and monocytes in clumps of these cells." He gives no criteria for separating this "monoblast" from the lymphocytes in the lymph node, although he denies that the lymphocytes give rise to the monocytes.

The previous investigators who used Bact. monocytogenes usually described the mesenteric lymph node after the infection had reached its height, in some cases as long as a month afterward. Bloom [2] reported the formation of monocytes in the mesenteric lymph node only in rabbits which had been splenectomized and subjected to injection of india ink into the portal vein and then injection of Bact. monocytogenes. In his normal rabbits after the injection of this bacterium he found that there were practically no monocytes or monocytoid cells in any parts of the mesenteric lymph nodes of rabbits receiving this bacterium alone or in conjunction with lithium carmine. It will be noted that all of his observations on the mesenteric lymph node were made either at the height of the monocytosis in the peripheral blood or subsequent to it, while my experiments have shown that the monocytogenic reaction in the mesenteric lymph node is at its peak prior to that of the monocytosis in the peripheral blood.

Witts and Webb,[12] using supravital preparations of material obtained by puncture of mesenteric lymph nodes, stated that the glands are absolutely destitute of monocytes and monoblasts; they examined the glands ten, twenty-four, forty-eight and seventy-two hours after the infection as well as later. Rezzesi,[5] using the same technic, commented on the fact that the mesenteric lymph nodes are not involved in the general monocytosis. Nyfeldt,[4] in his studies with a strain of Bact. monocytogenes isolated from a human being, agreed in substance with Bloom but described a great proliferation of large lymphoid cells in the mesenteric lymph node. However, he also concluded that this did not partake in the general monocytogenic response.

On the other hand, Levi and Penati [7b, c] studied the organs of rabbits infected with Bact. monocytogenes and described a great increase

10. Cunningham, R. S.; Sabin, F. R., and Doan, C. A.: Contrib. Embryol. **16**:227, 1925.

11. Forkner, C. E.: J. Exper. Med. **52**:385, 1930.

12. Witts, L. J., and Webb, R. A.: J. Path. & Bact. **30**:687, 1927.

in "lymphocytes and hemocytoblasts" in the mesenteric lymph nodes, as well as in the peripheral groups, prior to the peak of the monocytosis. But they concluded that the monocytes arise from the hemocytoblasts as a cell line apart from the lymphocytes, which are developing concomitantly. They base their conclusion that the monocytes are distinct from the lymphocytes on their study of dry smear preparations, stating that this differentiation is not possible in a study of sections or wet fixed smears. They do not, however, give the basis on which they have been able to discriminate between lymphocytes, hemocytoblasts and monoblasts in the dry smear preparations.

Throughout the course of my experiments the most striking feature in the early stages of the infection was the progressive increase, followed by a marked decrease, in the gross size of the mesenteric lymph node, together with the extensive cortical and medullary changes which I have described. This process is present not only in the rabbit, the animal used by the other investigators, but also, very prominently, in the guinea-pig. In rabbits and guinea-pigs killed at close intervals from the time of the injection to the peak of the monocytosis, the monocytes have been shown to develop by hypertrophy and transformation of lymphocytes. Complete series of cells intermediate between lymphocytes and monocytes are present as early as eighteen hours after the injection of Bact. monocytogenes and can be followed in sections as well as in dry and wet fixed smears of the mesenteric lymph nodes. Moreover, in the later stages of the infection in the guinea-pig the mesenteric lymph node, after the phase of extreme depletion, increases to a size far exceeding that of the control.

In the early stages of the infection in both the rabbit and the guinea-pig, the white pulp of the spleen and the cortex of the mesenteric lymph node show strikingly similar reactions. The participation of the spleen in the general monocytosis evoked by the bacterium is admitted by all, but the interpretations of this reaction are almost as numerous as there have been investigators. I believe the explanation of these divergences of opinion of the previous investigators of this subject is that all of them failed to study the tissues in the early stages of the infection.

In the usual defense reactions in the body, fixed macrophages become free and additional macrophages develop from lymphocytes. In focalized areas in heavy infection with Bact. monocytogenes, Bloom [13] described transformation of fixed macrophages into free phagocytes, but he saw no transformation of these macrophages or of the fixed cells into monocytes. In systemic infection with this bacterium, proliferation of the elements of the reticulo-endothelial system cannot be the source of

13. Bloom, W.: Arch. Path. **6**:995, 1928.

the monocytes that crowd the sinuses of the spleen and the lymph nodes. No evidence of the multiplication of reticulo-endothelial cells could be found in any of the early stages leading up the height of the monocytosis in the peripheral blood.

The diversified opinions as to the origin and nature of the monocyte have been reviewed by Maximow,[14] Bloom[15] and Levi and Penati.[7] A survey of the literature shows that the monocytes develop from lymphocytes or at least from cells that cannot be distinguished from lymphocytes by present methods. No one has given morphologic criteria by which all monoblasts can be distinguished from all lymphocytes (and myeloblasts). The separation of the cells on the basis of "their progeny" or of the "company which they keep" cannot be considered morphologic. The concept of a specific monoblast, i. e., a free cell morphologically different from a hemocytoblast, whether that be a myeloblast or a lymphocyte, has not been proved.

The conclusion presented here that the monocytes which are present in great numbers in infection with Bact. monocytogenes arise from lymphocytes is based on three main points: 1. The initial response to the injection of Bact. monocytogenes in both the rabbit and the guinea-pig is a mobilization of the lymphocytes present in the lymphatic tissue, followed by an intensive new formation of lymphocytes. 2. During the height of this initial hyperplasia, which precedes the peak of the monocytosis in the peripheral blood, all stages of transition between lymphocytes and monocytes are found throughout the diffuse and nodular lymphatic tissue of both the mesenteric lymph node and the spleen. 3. There is complete absence of any evidence of cells other than lymphocytes transforming into monocytes.

The sequence of events in the early stages in both the guinea-pig and the rabbit offers further proof of the correctness of the conclusion reached by Bloom[2] that "monoblasts other than lymphocytes do not exist."

CONCLUSIONS

The monocytosis evoked by infection with Bact. monocytogenes is essentially the same in rabbits and in guinea-pigs.

The initial stage in the production of the monocytosis is a mobilization of preexisting lymphocytes, followed by an extensive new formation of lymphocytes in the lymphatic nodules of the mesenteric lymph

14. Maximow, A.: Bindegewebe und blutbildende Gewebe, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 2, pt. 1, p. 232.

15. Bloom, W.: Folia haemat. **37**:63, 1928; Lymphocytes and Monocytes, in Downey, H.: Handbook of Hematology, Paul B. Hoeber, Inc., New York, 1937.

node and of the periarterial lymphatic tissue of the spleen. The peripheral nodes play a much less important part in the process.

This marked production of lymphocytes occurs in the early stages of the infection, before the height of the monocytosis in the peripheral blood.

The failure of previous investigators to study these early stages explains some of the differences of opinion among them.

The free stem cell from which the monocyte develops in the diffuse lymphatic tissue and lymphatic nodules of the mesenteric lymph node and in the spleen is morphologically identical with the lymphocytes in these tissues.

Monoblasts other than these lymphocytes have not been found.

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